

Figure 1 COLT-1 gene targeting vector and the structure of the COLIA1 targeted locus

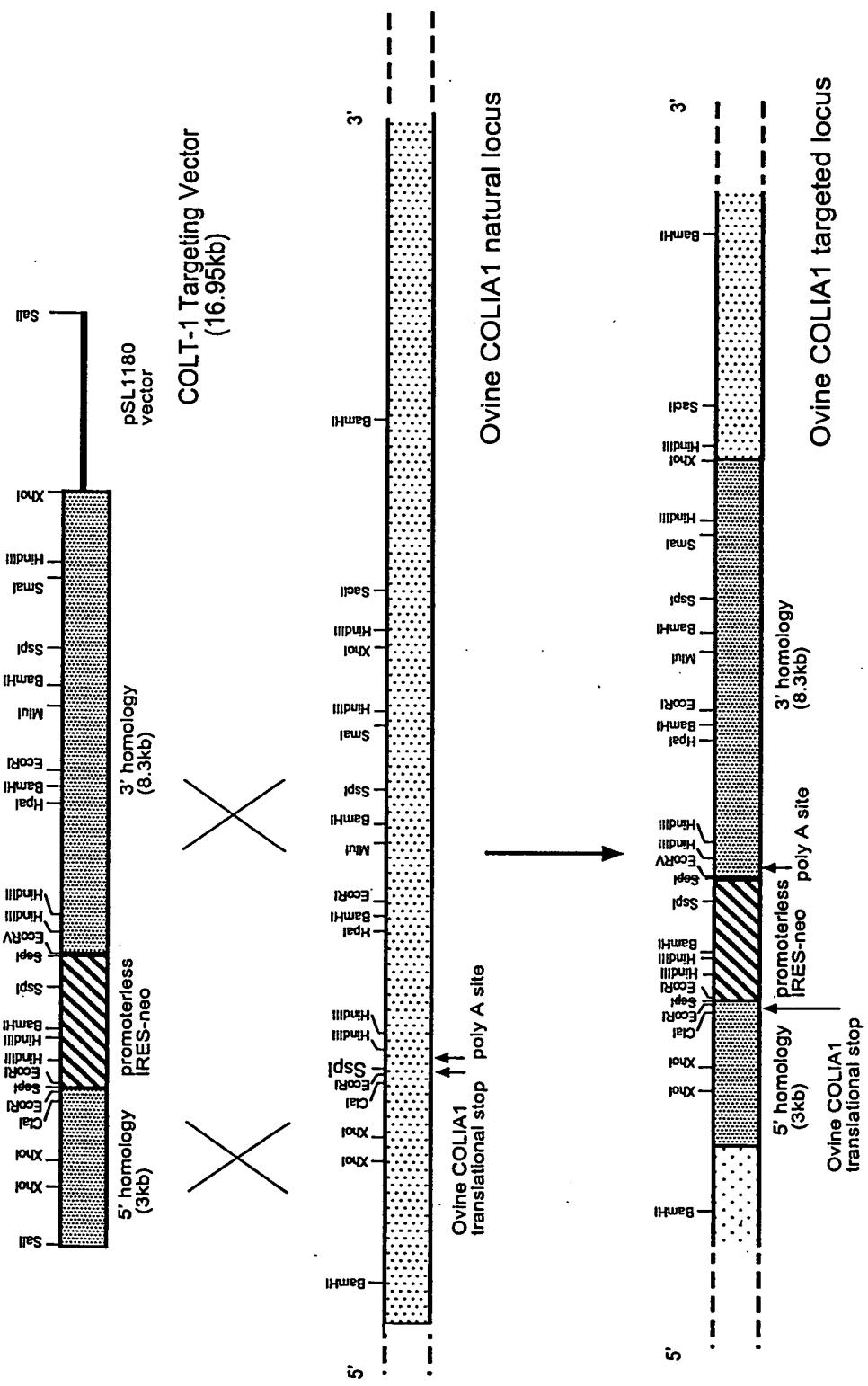
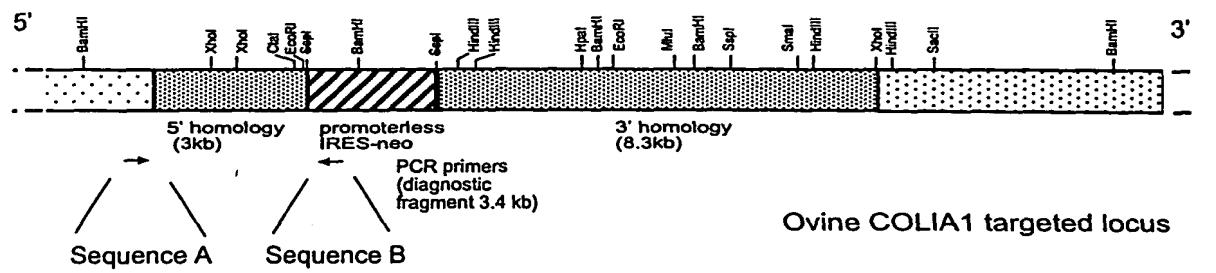


Figure 2. PCR screening strategy



Sequence A

Sequence A (5' homology region):

```

GAGCCACAGCTCAGCCTAAGGCCCTCCCCAGCCAGTACCCGTGTTCCCCAAGGAAGGGGTTGTCACCCAGCTACACAAAGCC
          ↓
          Primer COLT PCR4
          ↑
TAAATCTGCTTGAAGATTACCTGGGTCAAGGAGGGATGGATGTGGCAGGAACAGATGTGAAGGGATTGGCAAGGGAGATTCATCTGTAGCTCAGG
          ↓
          Msp20 I
          ↓
          BaI
          ↓
          MscI
          ↓
          Msp20 I
          ↓
          Bpu10 I
  
```

COLIA1 endogenous locus → COLT-1 5' homology

Sequence B

Sequence B (3' homology region):

```

TCGGCTTCGACATCGGCTCTGCTGCTTCCGTAAACTCCTTCCACCCAGCCTGGCTCCCTCCCACCCAACCCACTGGCCCTGACTCTGGAAACAGAC
          ↓
          Van91 I
          ↓
          Psp1406 I
          ↓
          COLT-1 5' homology
          ↓
          IRES neo
          ↓
          BspI
          ↓
          Avr II
          ↓
          Ppu10 I
          ↓
          BlnB I
          ↓
          Nde I
          ↓
          Nsi I
          ↓
          CclN I
          ↓
          Eco52 I
          ↓
          Not I
          ↓
          Xma III
          ↓
          Ecor I
          ↓
          Hha I
          ↓
          Ssp I
  
```

AATTCCGCCCTCTCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCCTTGCTATATGTTATTTCCACCATATTG
 ↓
 BseMI
 ↓
 BsrDI
 ↓
 Apa I
 ↓
 PpuI
 ↓
 Bbs I
 ↓
 BsaM I
 ↓
 Avr II
 ↓
 BsaM I
 ↓
 BsaM I
 ↓
 Primer COLT PCR8

Figure 3. PCR screen of COLT-1 PDFF2 transfectants



Figure 4. Sequence analysis at 5' junction of targeted locus

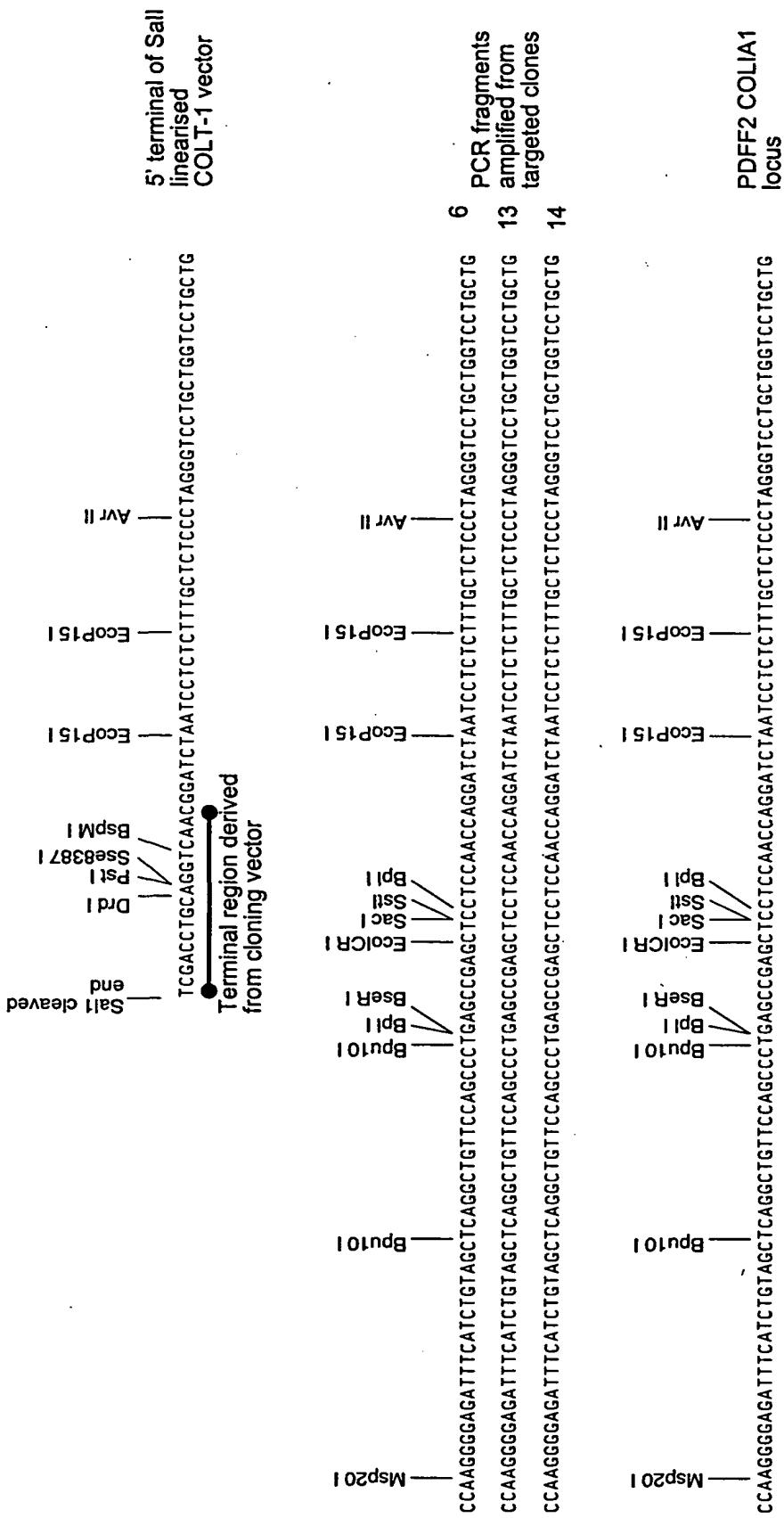
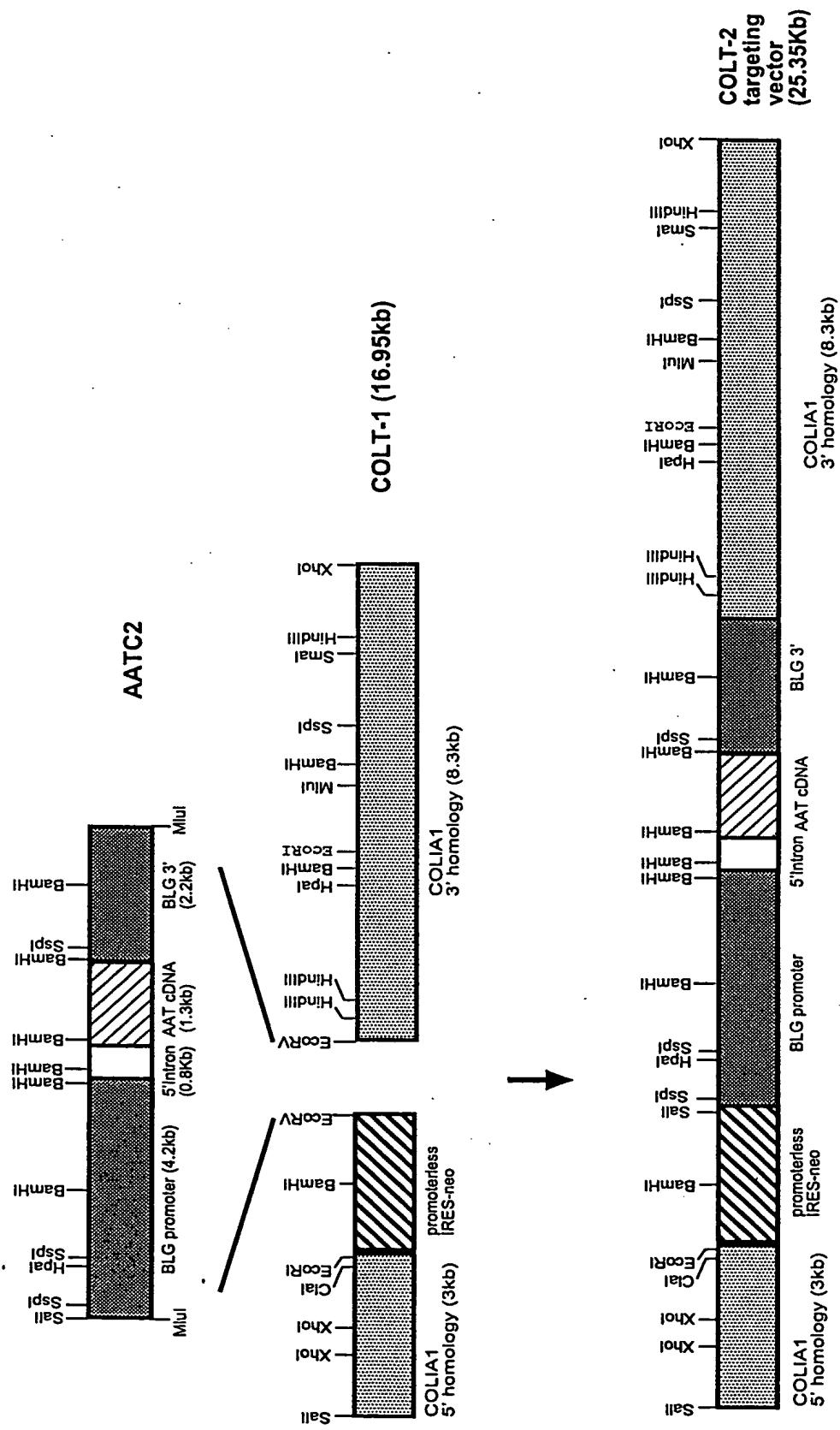
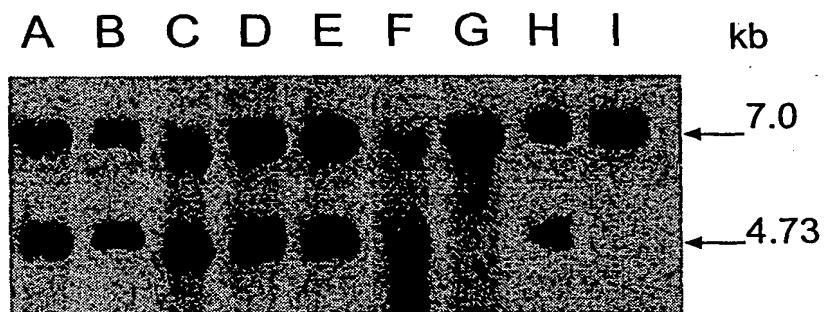


Figure 5. Construction of COLT-2 Transgene placement vector



100307-3-22502

Figure 6. Southern analysis of gene targeted lambs



Key to lanes

COLT1 targeted lambs
(nuclear transfer from cell clone PDCOL6)
A. live lamb born from ewe 941235
B. stillborn lamb from ewe 9600356
C. live lamb born from ewe 950030
D. stillborn lamb from ewe 941053.

COLT 2 targeted lambs
(nuclear transfer from cell clone PDCAAT90)
E. stillborn lamb from ewe NZ1418
F. live lamb born from ewe 941026
G. wild type lamb
H. cell clone PDCAAT90
I. non transfected PDFF2 cells

Figure 7. Structure of the COLT-1 and COLT-2 targeted loci

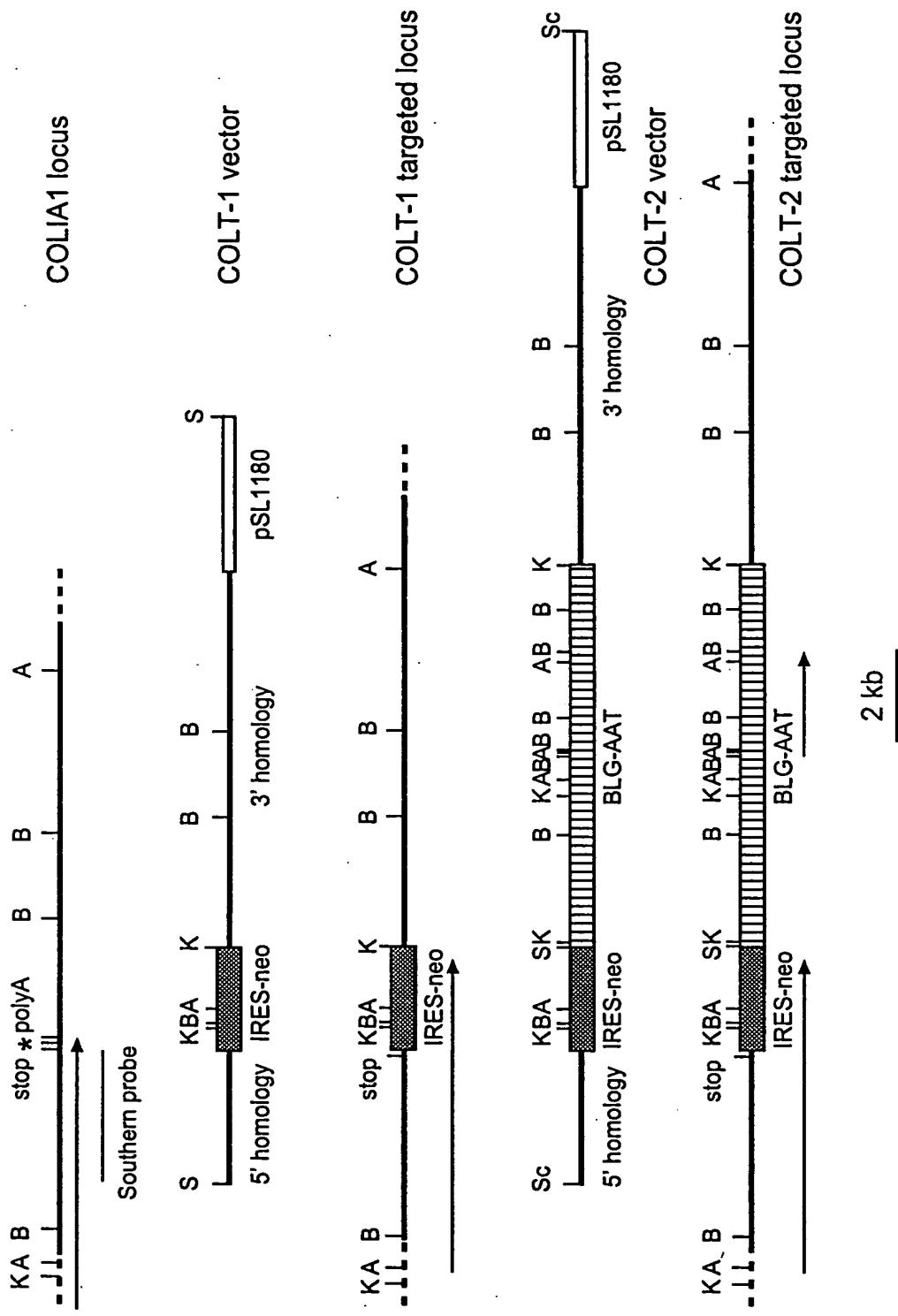


Figure 8. Southern analysis of COLT-2 transfected (PDCAAT) cell clones

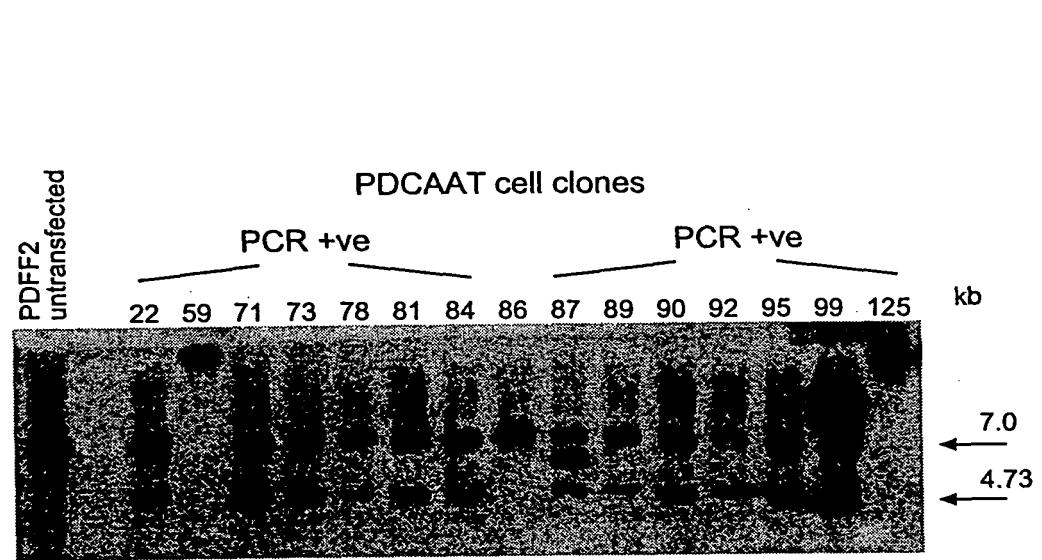


Figure 9. Northern analysis of COLT-2 targeted (PDCAAT) cell clones

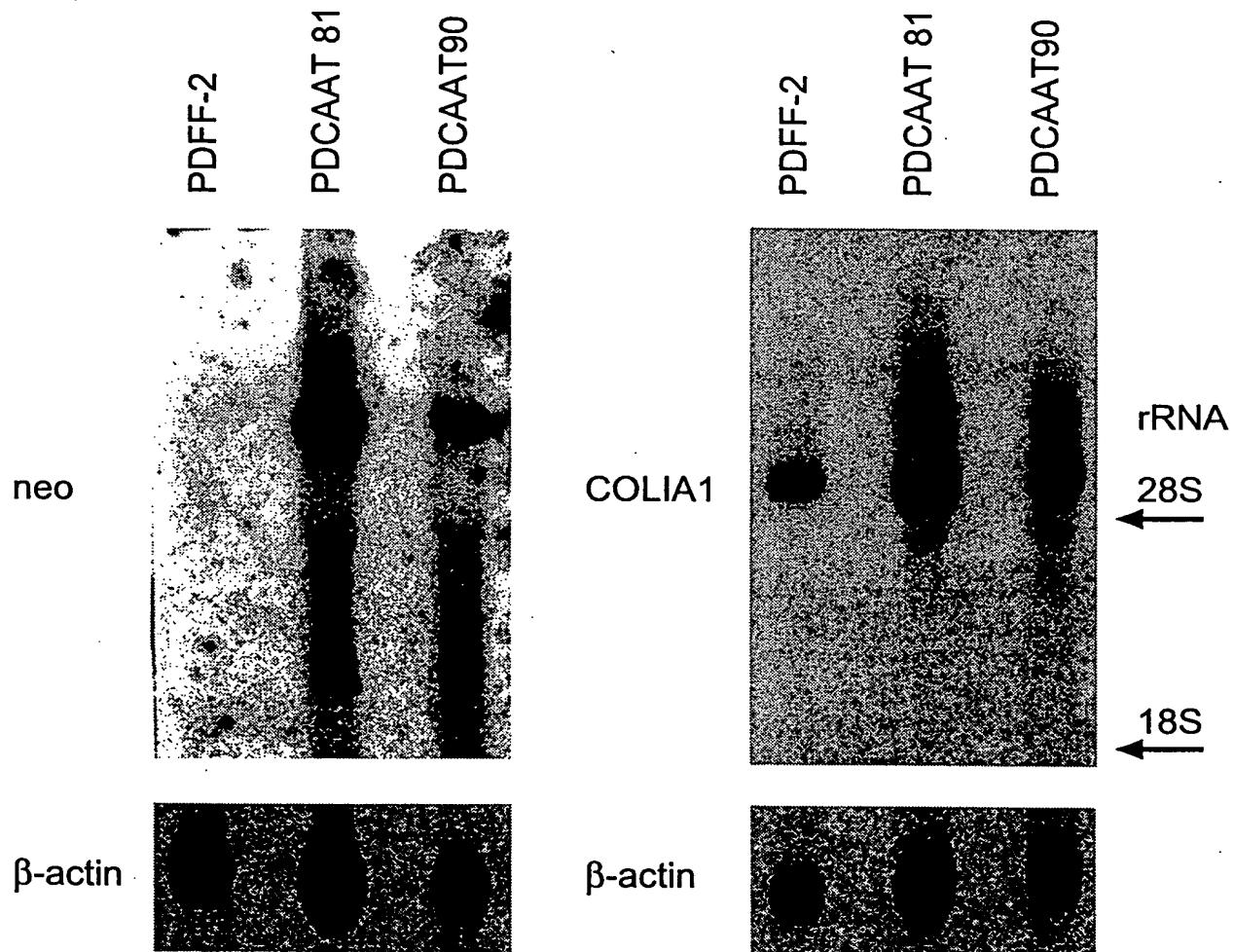
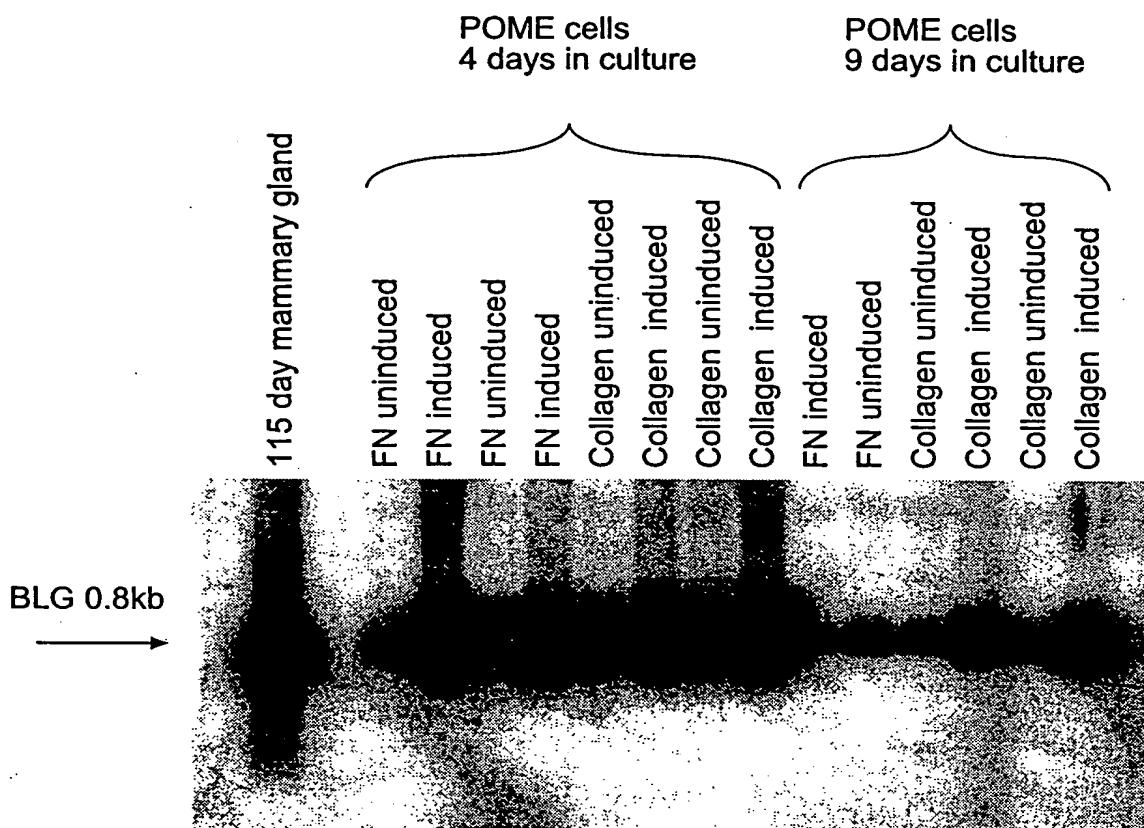


Figure 10. Northern analysis of primary ovine mammary cells in culture



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Figure 11. Ovine beta lactoglobulin targeting vector BLAT-3

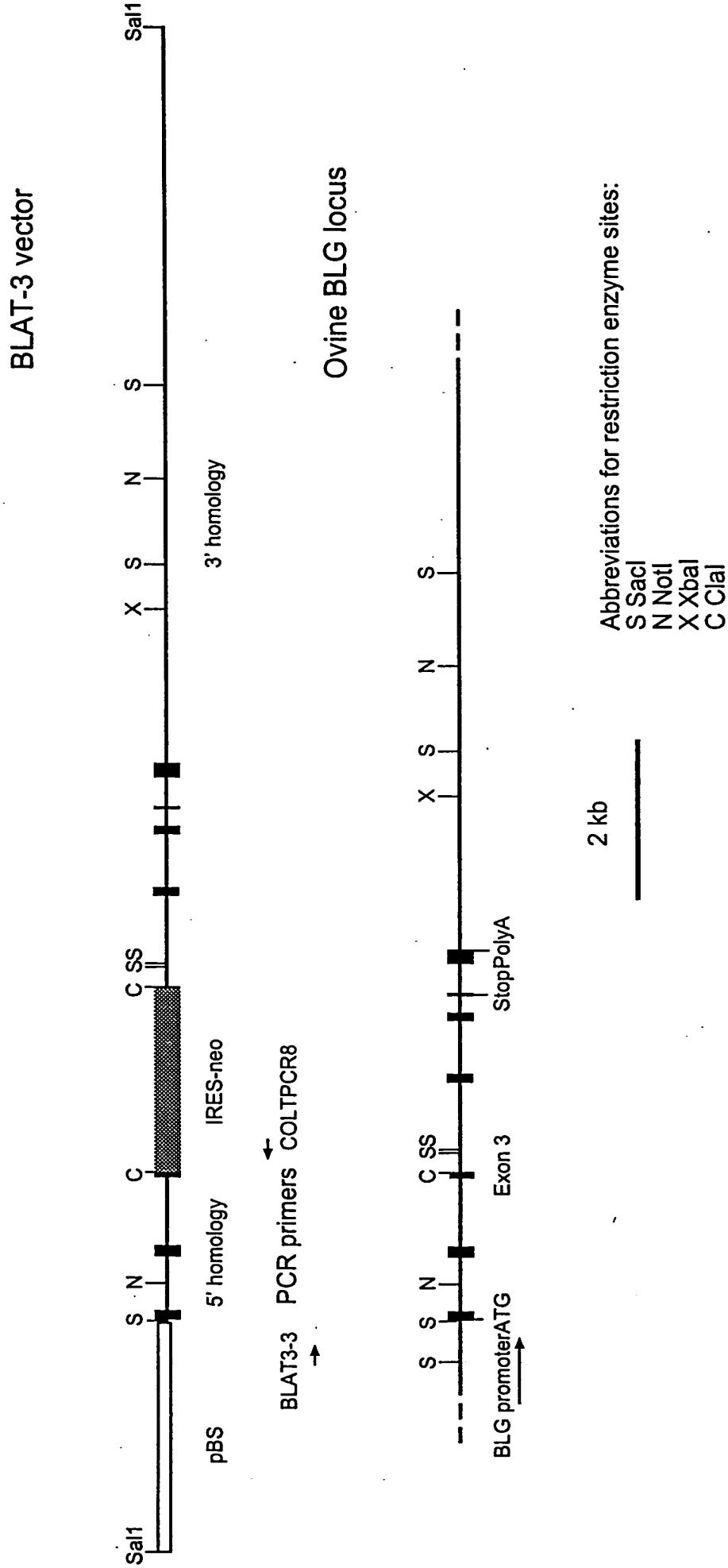
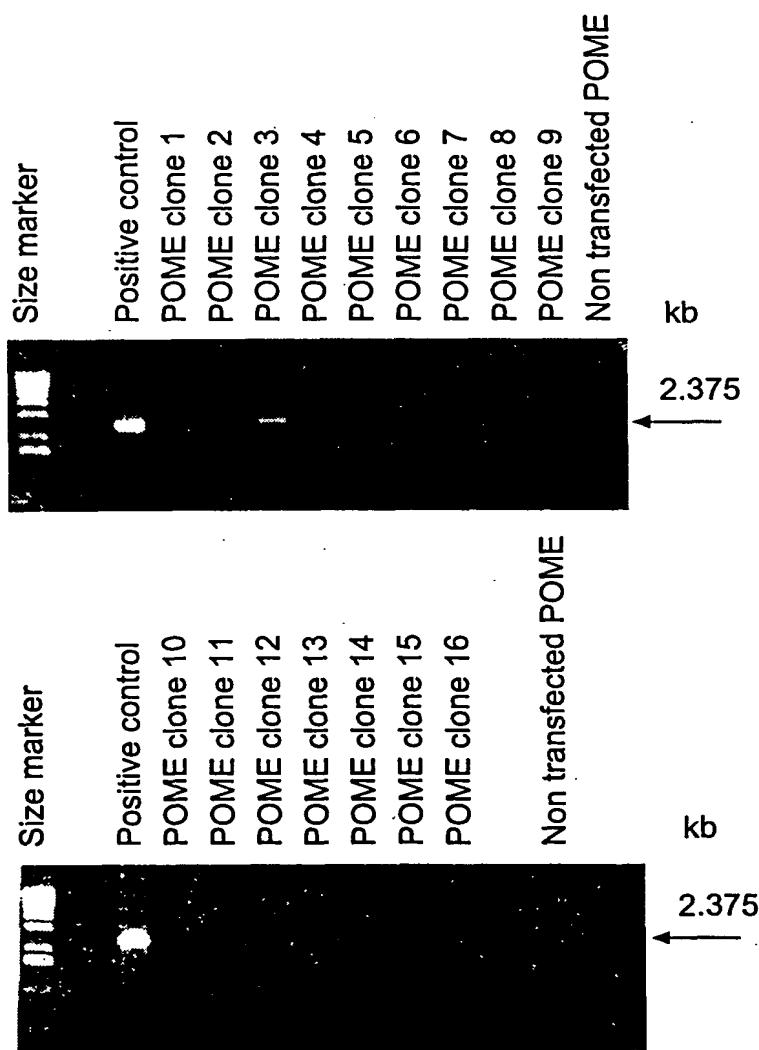


Figure 12. PCR analysis of BLAT-3 transfected POME cell clones



**Figure 13. Promoter-trap Knockout Vector for Porcine
α-1,3-Galactosyltransferase Gene**

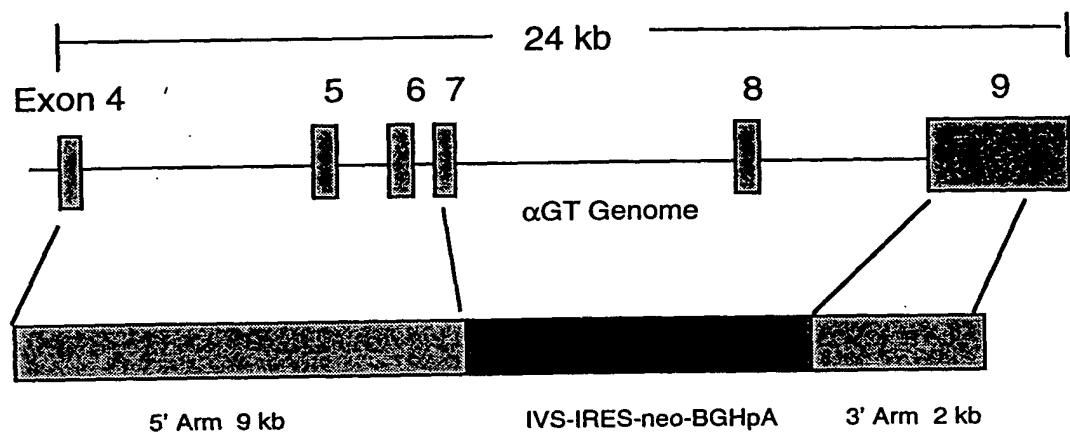


Figure 14. PCR Screening and DNA Sequencing of α GT Knockout clones

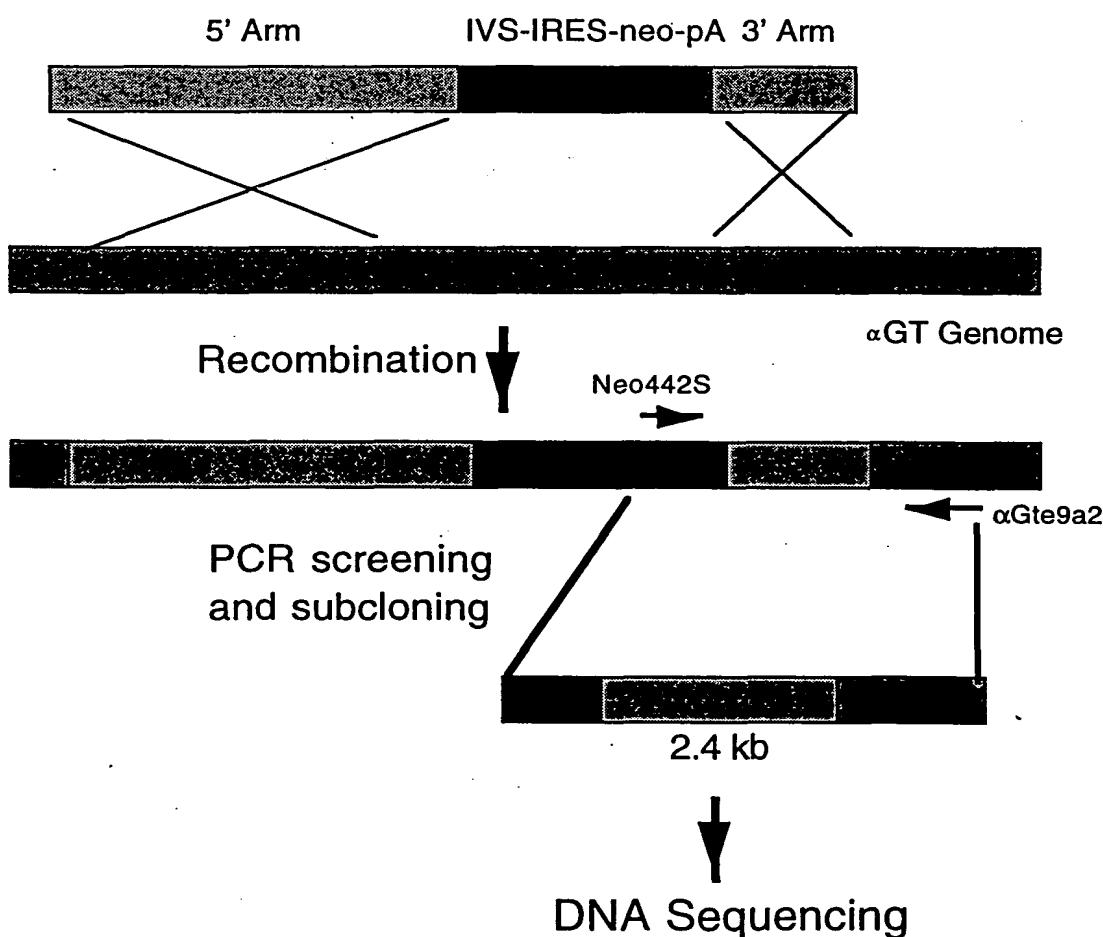


Figure 15. Sequence analysis over 3' junction region of PCR fragment amplified from porcine α -1,3GT targeted locus

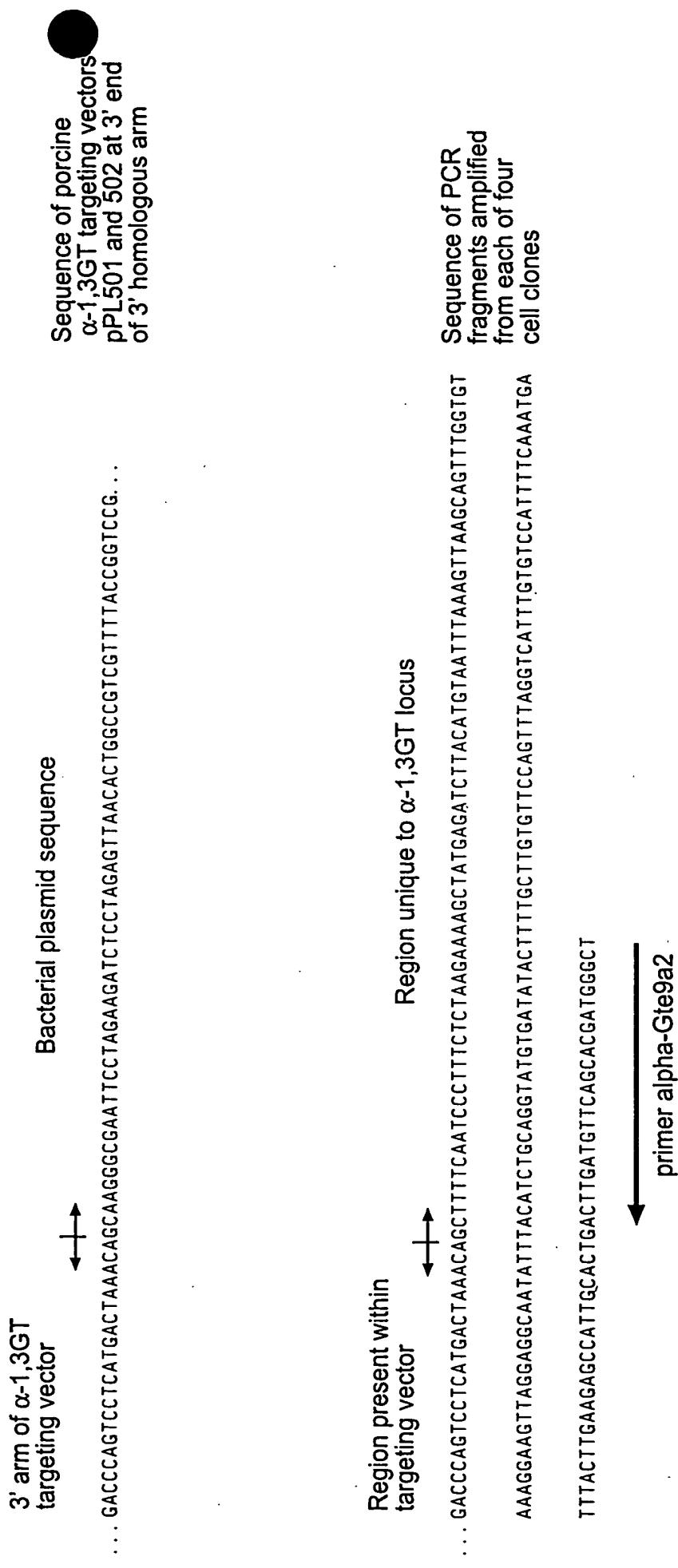


Figure 16. Poly A-trap Bovine BLG Knockout Vector

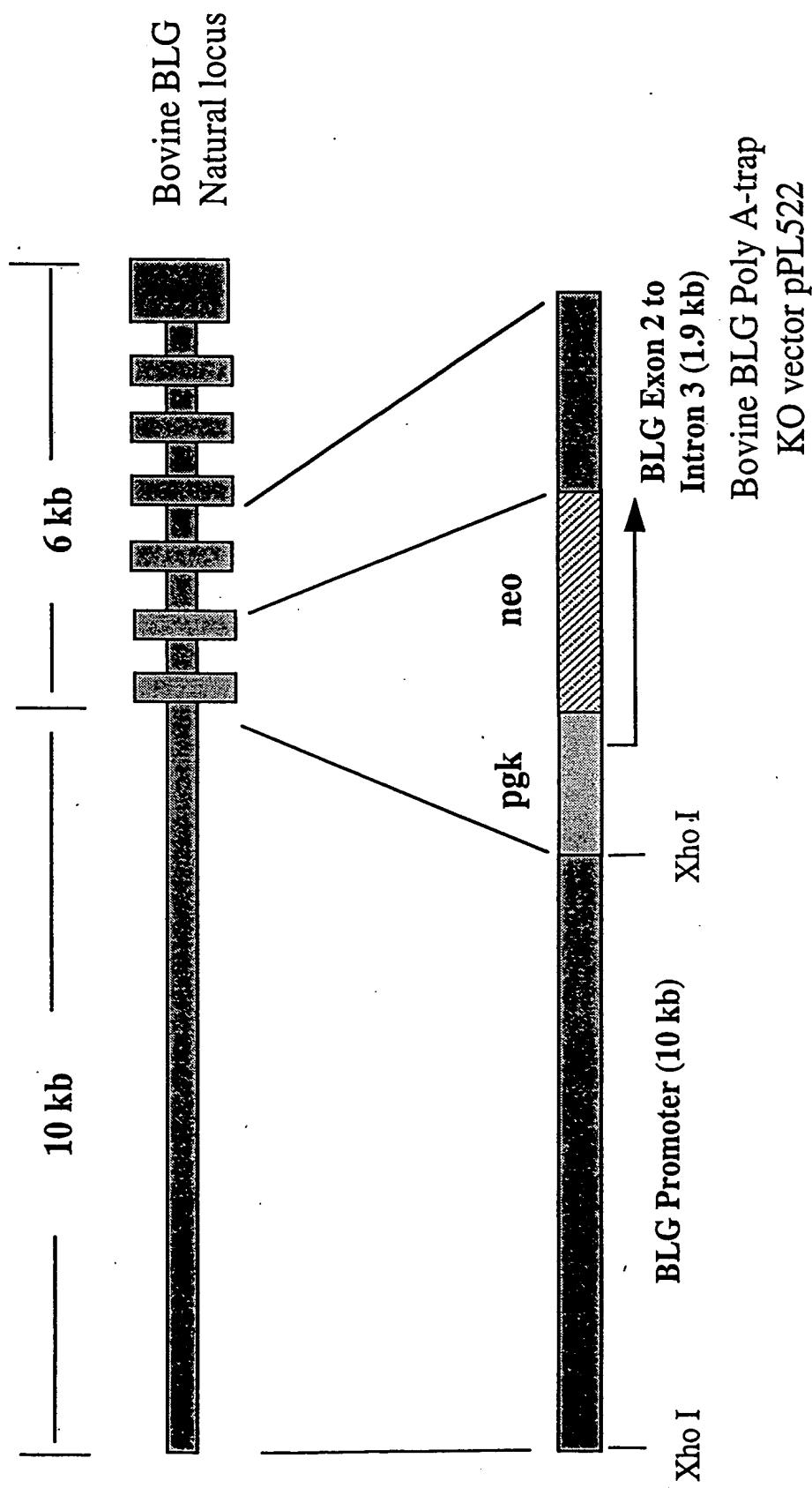
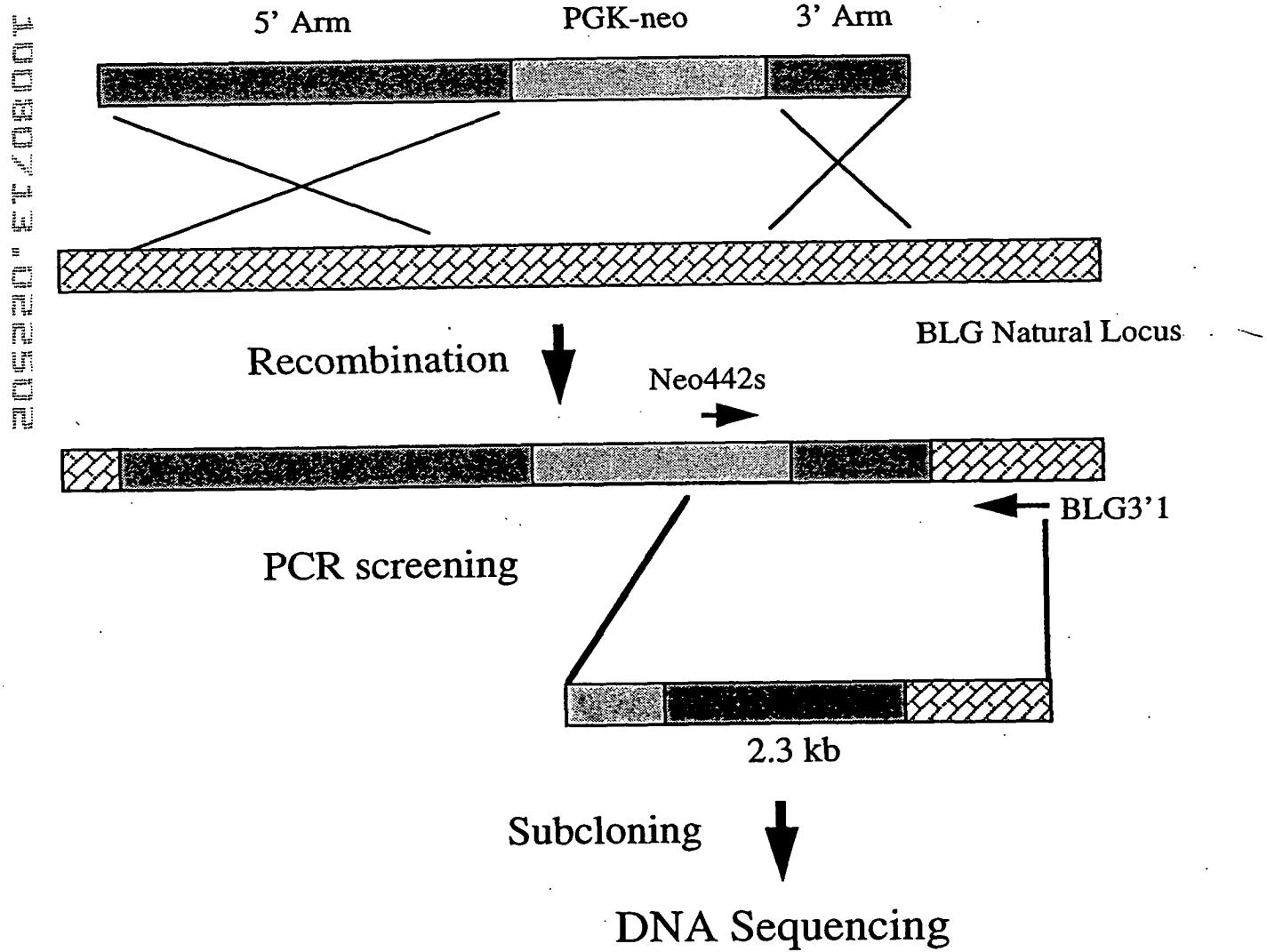


Figure 17. PCR Screening and DNA Sequencing of BLG Knockout clones



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Figure 18. Sequence analysis over 3' junction region of PCR fragment amplified from bovine BLG targeted locus

